

Title Quantitative expression of *Paracoccidioides brasiliensis* secreted aspartyl protease gene *PbSAP* after dimorphic transition and different stress conditions.

Authors Castilho, D. G.¹; Chaves, A. F. A.¹, Navarro, M. V.¹, Calado, J. C. P.¹, Conceição, P. M.², Xander, P.², Batista, W. L.^{1,2}

Institution ¹Departamento de Microbiologia, Imunologia e Parasitologia/UNIFESP-São Paulo; ²Departamento de Ciências Biológicas/UNIFESP-Diadema, Brazil.

Abstract:

Paracoccidioides brasiliensis and *P. lutzii* are the agents of paracoccidioidomycosis, a systemic mycosis endemic in Latin America. The infection begins after inhalation of the fungal propagules and their thermo-dimorphic shift to yeast form. The development of the disease depends on factors associated with the host immune response and the infectious agent characteristics. Aspartyl proteases (Sap) are virulence regulators in others pathogenic fungi and play an important role in the host invasion process. In a comparative quantitative proteomics study (LC-MS/MS), we showed that the protease was overexpressed in virulent *P. brasiliensis* isolated (vPb18) compared to attenuated isolated (aPb18). The present work aimed to evaluate the *PbSAP* gene expression after passages of aPb18 through mice infection, during dimorphic transition and after fungal treatment with different stress conditions. The *PbSAP* expression was evaluated by qRT-PCR after two passages of attenuated fungus in mice. The gene encoding *PbSAP* showed an increase (2-fold) in their expression after the recovery of virulence of attenuated fungus. The transcription level of *PbSAP* was also evaluated in fungal treated under stress conditions including oxidative, osmotic and heat stress. The *PbSAP* expression showed an increase in early oxidative (2 h at 2 mM H₂O₂) (2.6-fold) stress, which was not observed after later periods (6 h). Under osmotic stress, at 1 mM NaCl, was observed a raised in the mRNA expression levels of *PbSAP* gene (2.5-fold). Interestingly, after treatment for 1 h at 42°C the expression of the same gene was 40-fold higher compared to the control. In addition, the transcription level of *PbSAP* was evaluated during the dimorphic mycelium-yeast-mycelium (M-Y-M) transition. In the M-Y transition, there was increase of about 23-fold at 5 h, followed by constant levels of expression and a small decrease after 120 h. Still, there was a 4 to 15-fold decrease in the mRNA expression during the Y-M transition. Our results shown that the *PbSAP* gene is differentially expressed in the transition, with a progressive increase in *PbSAP* expression during the period of conversion from M-Y. Therefore, these results suggest that the *PbSAP* gene may be required both for response to different stress conditions as for fungal survival in the host environment during early response.

Key words: *Paracoccidioides brasiliensis*, virulence, aspartil protease

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